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KTP LASER-INDUCED BIOMODULATION OF THE WOUND-HEALING PROCESS

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ABSTRACT

The KTP laser (wavelength, 532 nm) was used in a subablative format to determine whether low energy density irradiation would affect the normal healing by primary intention of scalpel skin incisions in rats. Two longitudinal lased strips were created by a 1 cm diameter defocused beam on the shaved, cleaned dorsal epidermis of 32 Sprague-Dawley rats; one strip was produced with a 2.0 W beam (54 J, or 18 J/cm^2 total dose), and the other with a 3.5 W beam (94.5 J, or 31.5 J/cm^2, total dose). Scalpel incisions were made longitudinally within the irradiated zones, using contralateral scalpel incisions on unirradiated skin as controls. Tensiometric analysis of wound strength was performed at 3, 7, 14, and 23 days following surgery. The data from fresh tissue tensiometry indicate that KTP laser irradiation of skin incisions results in a triphasic tensile strength response for the 54 J (2.0 W) exposure, but not for the 94.5 J (3.5 W) exposure. For the 54 J irradiated incisions, the fresh tensile strength is greater than control at days 3 and 23, but less than control at the intermediate days 7 and 14. The 94.5 J irradiated incisions are consistently weaker than controls. These effects may be explained by the interplay of localized transient cellular injury effected by the low energy density laser and a concomitant biomodulatory response.

INTRODUCTION

Medical lasers have traditionally been used for tissue ablation since laser energy can cause intense localized heating sufficient to vaporize both extra- and intracellular water, producing a coagulative necrosis.[1-3] The carbon-dioxide (CO_2) laser is often the laser of choice when laser incision and tissue ablation are desired, but the hemostatic effects are suboptimal.[3,4] Other lasers, such as the KTP and argon (wavelengths of 532 nm and 488 nm, respectively), are much more effective at hemostasis since their frequencies of emission lie near the absorption maximum of hemoglobin. Any of these lasers, especially the CO_2, may be expected to produce thermal tissue damage lateral to the site of irradiation when used in the ablative energy range.[5] This lateral damage may be responsible for the well-known delay in the healing of laser incisions.[1,6]

More recent literature has begun to focus on the non-thermal, or photochemical, property of the laser and its use in the biomodulation of physiologic processes.[5,7-12] Both the helium-neon and argon lasers, when operated in a subablative format, have been shown to augment the rate of collagen synthesis and the total collagen deposition in skin wounds,[7] but an attenuated rate of wound reepithelialization was documented when using the CO_2 laser.[5] Unfortunately, however, the degree to which photochemical and thermal processes contribute to the biomodulation is not easily delineated, although it is predicted that photochemical processes are favored at lower

irradiences. We investigated the effect of low energy density KTP irradiation on the normal wound healing process of skin incisions in rats, and our results suggest that the KTP laser can be utilized as a biomodulatory tool.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 275-300g initially, were anesthetized intraperitoneally with Ketaset (ketamine hydrochloride, 22.0 mg/kg body weight; Aveco, Co., Inc., Fort Dodge, IA) and Rompun (xylazine, 2.2 mg/kg body weight; Haver, Mobay Corp., Shawnee, KS). With the onset of analgesia, the dorsal pelt of each animal was clipped (Model #5-01, Head #80, size 10; Oster, Inc., Allegheny International Co., Professional Products Department, Milwaukee, WI), shaved, and the exposed dorsal epidermis was treated with a depilatory (Magic Depilatory Cream, Carson Products Co., Savannah, GA) to remove any residual stubble which would interfere with absorption of the laser. Each rat was mounted on a 20.4 cm X 20.4 cm plexiglass translation stage. The stage was propelled horizontally at a constant rate of 0.11 cm/sec via its attachment to both pistons of a Harvard pump, and the translation stage was positioned immediately beneath the vertical KTP beam (KTP/532 Laser, Serial #1605246, Laserscope, Santa Clara, CA). The Laserscope KTP is a quasi-continuous wave laser whose pulse regime consists of a 25 kHz pulse repetition rate with a 1 μsec pulse width; the beam profile, as determined by another member of our department (see Acknowledgments), was shown to be near Gaussian.

The KTP beam was defocused to a diameter of 1 cm through the use of a Microbeam 1 (Laserscope, Santa Clara, CA) attachment on a Storz (Model US-1,M1061-50/50, Storz Urban, Urban Engineering, Inc., Burbank, CA) operating

microscope, the vertical distance from the beam orifice to the rat's dorsum being 18 cm. Each rat was translated under a 2.0 W KTP beam for a duration of 27 sec (54 J, or 18 J/cm^2, total dose) to produce a 3 cm longitudinal strip 1.5 cm to the left of the spinal column. A second 3 cm lased longitudinal strip was created ipsilateral and 2 cm posterior to the first strip by using a 94.5 J (3.5 W, 31.5 J/cm) KTP beam. The total doses delivered were 18.0 J/cm^2 for the 2.0 W exposure and 31.5 J/cm^2 for the 3.5 W exposure. These two doses were chosen empirically as a result of a pilot study involving one rat which was exposed to a range of power settings (0.5 W to 15 W) at the corresponding doses of 1.9 J/cm^2 to 57.3 J/cm^2: the observations lead us to select two levels of irradience, one of which (54 J) produced no evidence of erythema or induration and the other of which (94.5 J) was just sufficient to produce visible erythema and induration. These irradiences were termed maximally sublethal and minimally lethal, respectively, although these terms were chosen only for descriptive purposes.

Immediately subsequent to the irradiation procedure, 3 cm longitudinal scalpel incisions were created with a No. 20 blade (SteriSharps, Seamless Hospital Products Co., Wallingford, CT) in the midline of each of the lased strips. Control scalpel incisions were created contralaterally at identical vertebral levels. All scalpel incisions extended down to the panniculus carnosus, and each incision was closed with four skin staples following careful apposition of the skin flaps. The rats were then housed in individual cages without wood shavings (to keep the wounds as clean as possible), with water and chow (Wayne Feeds, Precision Milling, Chicago, IL) *ad libitum*, and on a 12 h light / 12 h dark cycle *per diem*. After excluding one rat which was sacrificed acutely for histology, each of the

remaining rats was randomly allocated to one of four recovery groups: the groups were terminated at 3, 7, 14, and 23 days postoperatively.

On the designated days the rats were sacrificed by a lethal dose of inhaled halothane (containing 0.01% Thymol, Halocarbon Laboratories, Inc., Hackensack, NJ), and a 2.5 to 3.5 cm X 2.0 to 3.0 cm skin patch containing the wound was excised using a No. 20 scalpel blade. A 1 cm X 2 to 3 cm block was removed from one end of the initial patch to be processed for routine light microscopy. The remaining patch (1.5 to 2.5 cm X 2.0 to 3.0 cm) was placed in PBS buffer [8.1 mM Na_2HPO_4, 0.5 mM $MgCl_2$ (all obtained from Fisher Scientific, Fair Lawn, NJ) in sterile saline (0.9% NaCl, Travenol Laboratories, Inc., Deerfield, IL)] and chilled on ice until fresh-tissue tensiometry was performed, which was within 1.5 hours in all cases. All of the wounds were excised in the above manner, and extreme care was taken to avoid exerting any stress on the wound prior to its tensiometric measurements. In an effort to aid in this respect, the skin staples were not removed until immediately prior to placing the skin patches in PBS buffer.

Each of the skin patches was positioned in the jaws of the tensiometer (Instron, Model 1130, Instron, Inc., Canton, MA) which was equipped with a 5000g head; the patch was oriented with the scalpel incision parallel to the edge of the tensiometer's jaw. In the event that the skin patch was dislodged from the jaws during tensiometry, the measurement was aborted. Peak breaking forces were measured, and the forces were converted to strain values (g force/incision length) based on the length of the incision.

Statistical Method

The tensiometric data were transferred manually from tensiograms to an IBM Turbo AT computer operating with the statistical software SYSTAT (SYSTAT, Inc., Evanston, IL). This software package was then utilized to analyze the data and calculate confidence intervals based on the Wilcoxon signed-rank test.

RESULTS

Tensiometry

All wounds showed a time-dependent increase in tensile strength over the 23 day period (refer to Table I). However, the wounds which had been irradiated with 54 J (2.0 W beam) showed a triphasic tensile strength response. Figure 1 illustrates that at postoperative day 3, the irradiated incisions were **stronger** than control incisions by 59% ($p \leq 0.05$). However, for postoperative days 7 and 14, the irradiated incisions were weaker by 44% and 19%, respectively ($p \leq 0.01$ for both days). Then by postoperative day 23, the strength of the irradiated incisions has again superceded that of controls by 29% at the 90% confidence limit. The incisions which were irradiated with 94.5 J (3.5 W beam) were consistently weaker than those of controls, as depicted in Figure 2, although only the point at day 14 is statistically significant within the 95% confidence interval. The points at days 7 and 23 are, however, suggestive of reduced tensile strength in the 94.5 J (3.5 W) irradiated incisions.

Histology

Analysis of the tissue sections revealed several remarkable findings. In the animal which was acutely sacrificed, the 54 J (2.0 W) irradiated wound showed no destruction of the epidermis or subjacent dermal stroma, no evidence of tissue edema, and was identical to control (refer to Figures 3 and 4). The 94.5 J (3.5 W) irradiated wound, however, showed a zone of thermal disruption of the dermal collagenous

stroma to a depth of 0.6 mm, leaving the reticular dermis unaffected; substantial tissue edema was noted (refer to Figure 5). On postoperative day 3, an augmented coagulum was seen in the 54 J (2.0 W) irradiated wound as compared to control, accompanied by a substantial inflammatory cell infiltrate throughout the dermis (refer to Figures 6 and 7). Furthermore, the epithelial bridging of the incision appeared to be attenuated. The sections for the remaining days of the study were unremarkable except that reepithelialization was retarded for both 54 J and 94.5 J irradiations until day 14. Dermal collagen content and organization appeared to be similar in irradiated and control wounds for all days surveyed, but visual quantitation is highly subjective and unreliable.

DISCUSSION

Recent literature in the field of medical application of laser irradiation suggests that some of the currently used medical lasers may be utilized for purposes other than mere tissue ablation.[7-14,17,18] Laser-mediated biomodulation of tissue, either *in vitro* or *in vivo*, is a new field of investigation in which the laser is operated at energy densities which are insufficient to effect tissue vaporization, but which appear to be sufficient to cause both biostimulation and bioinhibition. Both types of modulation appear to have some energy density and wavelength specificity.[9,12,15,17]

In this study we investigated the potential use of the KTP laser (532 nm wavelength) as a biomodulatory tool when operated in a subablative format. Our tensiometric data indicate that the KTP laser can be used to alter the normal healing by primary intention of skin wounds. The distinctly different results obtained from the 54 J (2.0 W, 18 J/cm^2) and 94.5 J (3.5 W, 31.5 J/cm^2) irradiations show that the tissue response is indeed energy density dependent. The 54 J irradiation produced a triphasic tensile strength response as evinced by an initial increase in tensile strength over control at day 3, followed by a decreased tensile strength as compared to control, and concluded by another increase in tensile strength by postoperative day 23. Indeed, the initial increase in tensile strength at postoperative day 3 for the 54 J irradiation could be explained by local vascular injury resulting in increased vascular permeability with a resultant increase in fibrin exudation; this hypothesis is supported by the presence of

an increased coagulum in the 54 J wound at day 3. The reduction in tensile strength of the 54 J irradiated wounds on days 7 and 14 is probably best explained by a transient cellular injury causing the release of proteases which may delay the reparative process, and by the attenuated reepithelialization of the irradiated incisions (refer to Histology Results). However, under this schema the eventual rise in tensile strength over control by day 23 is enigmatic unless one also posits a concomitant biostimulatory effect of the laser. In contrast, the 94.5 J (3.5 W, 31.5 J/cm^2) irradiation did not produce a triphasic response. Instead, these irradiated wounds were consistently weaker than their matched controls, even after 23 days of recuperation, perhaps as a result of irreparable cellular and/or microvascular damage.

Other investigators have reported that the helium-neon laser has biostimulatory effects on collagen synthesis in wound healing as early as 3 to 4 days postoperatively.[13,14] As we have demonstrated, however, the response to 54 J (2.0 W) KTP radiation can not be explained mechanistically in such simple terms. If the observed response is due to laser biostimulation of collagen synthesis in the wound, such a stimulation would then necessarily be followed by either a resultant increase in collagenase activity or a decrease in collagen synthetic activity, or both: this scenario would be in agreement with our observations, but upregulation of collagen synthesis is not expected as early as three days postoperatively. Alternatively, the 54 J (2.0 W) irradiation may cause the local transient release of various tissue trophic factors such as epidermal growth factor (EGF) or transforming growth factor-*beta* (TGF-β) or various mediators of the inflammatory response such as interleukin-1 (IL-1) and other chemoattractants; such a local release may be explained by local microvascular changes, especially with regard to the endothelium, which are mediated by intense hemoglobin

absorption of the KTP beam. While these various tissue factors may be released early after laser exposure, their biomodulatory effects may be initially masked by protease release from transiently injured cells, only to be manifested once these cells have resumed their normal metabolism. The consistently weaker wounds for the 94.5 J (3.5 W) irradiation could be explained by irreparable microvascular and cellular damage to the area, causing a paucity of nutrients, growth factors, and inflammatory cells necessary to promulgate expedient healing.

We have shown that the KTP laser can be used in a subablative format to effect alterations in normal wound-healing physiology. The changes which were noted on routine light microscopy include thermal disruption of the dermal collagenous stroma in 94.5 J (3.5 W) irradiated wounds, increased wound coagula through day 7 for the 54 J (2.0 W) irradiated wounds, and attenuated reepithelialization through day 14 for both irradiences. The precise molecular mechanisms accounting for the observed histologic and tensiometric changes remain obscure. In order to elucidate these mechanisms, one must employ molecular probing techniques for biomolecules such as messenger RNAs (ribonucleic acids) for collagen, IL-1, EGF, and TGF-β, which are known to have pivotal roles in wound healing. Such probing techniques afford the localization and quantitation of these molecules within the tissue. Future studies of this sort are planned in an effort to characterize more precisely the biologic response to the KTP laser.

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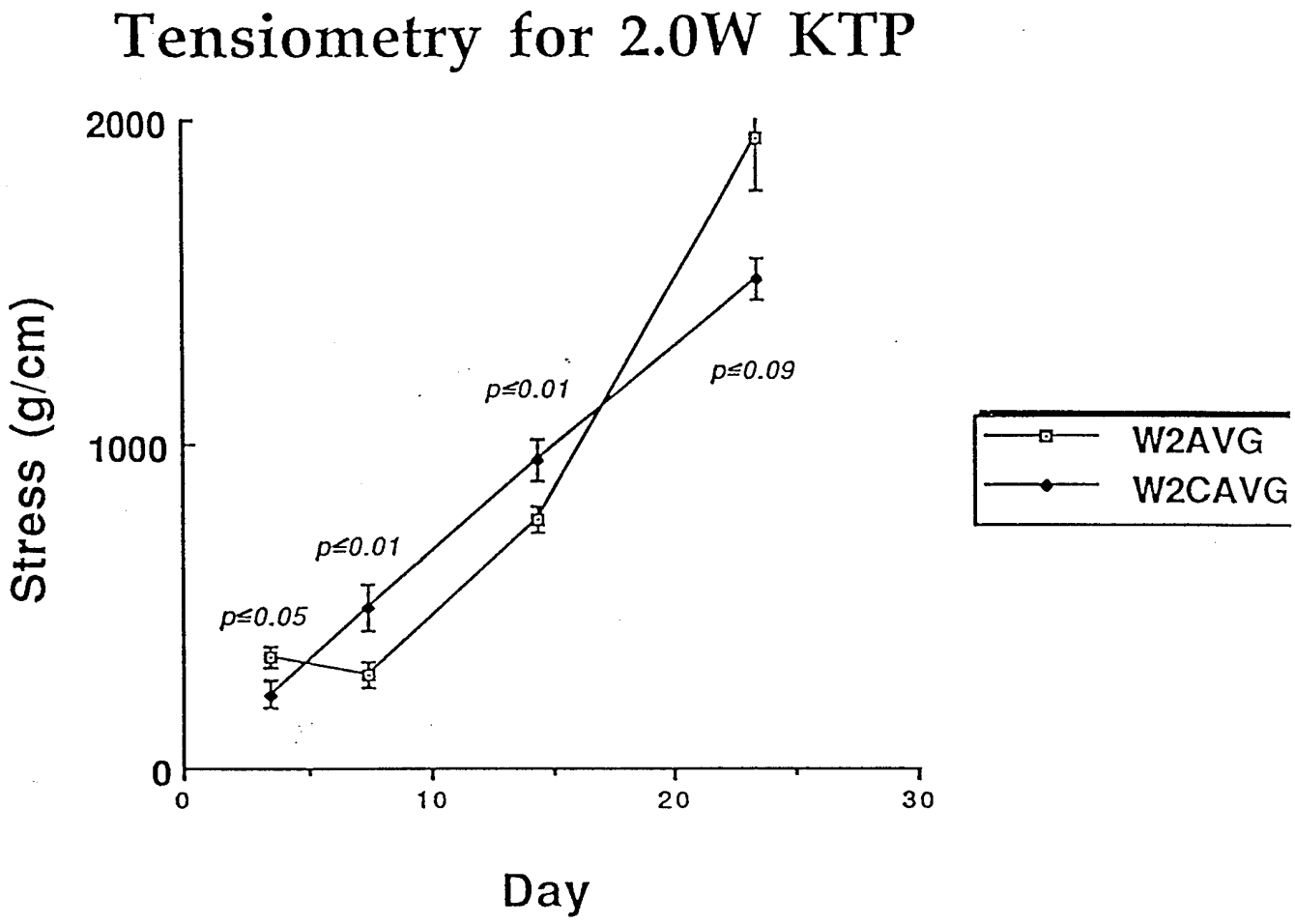
Table 1. Tensiometric data for laser irradiated incisions and their respective controls.

Postoperative Day	2.0W Irradiation	2.0W Control	3.5W Irradiation	3.5W Control
3	317.7 ± 34.0 (n=7)	200.2 ± 44.2 (n=9)	241.9 ± 18.2 (n=7)	262.2 ± 15.5 (n=9)
7	263.9 ± 39.5 (n=8)	470.2 ± 69.1 (n=8)	276.8 ± 38.4 (n=8)	472.2 ± 53.1 (n=8)
14	743.9 ± 40.3 (n=8)	921.1 ± 63.4 (n=7)	655.1 ± 41.9 (n=8)	854.9 ± 68.1 (n=8)
23	1909 ± 154 (n=8)	1480 ± 63.3 (n=5)	1689 ± 207 (n=8)	2145 ± 194 (n=7)

LEGEND FOR TABLE 1

The values represent average breaking strain (g force per cm incision length) for the indicated incisions. The numbers in parentheses represent the number of animals used per trial, and the tolerance values are standard errors of the means. Controls are unirradiated scalpel incisions on the contralateral side of the animal.

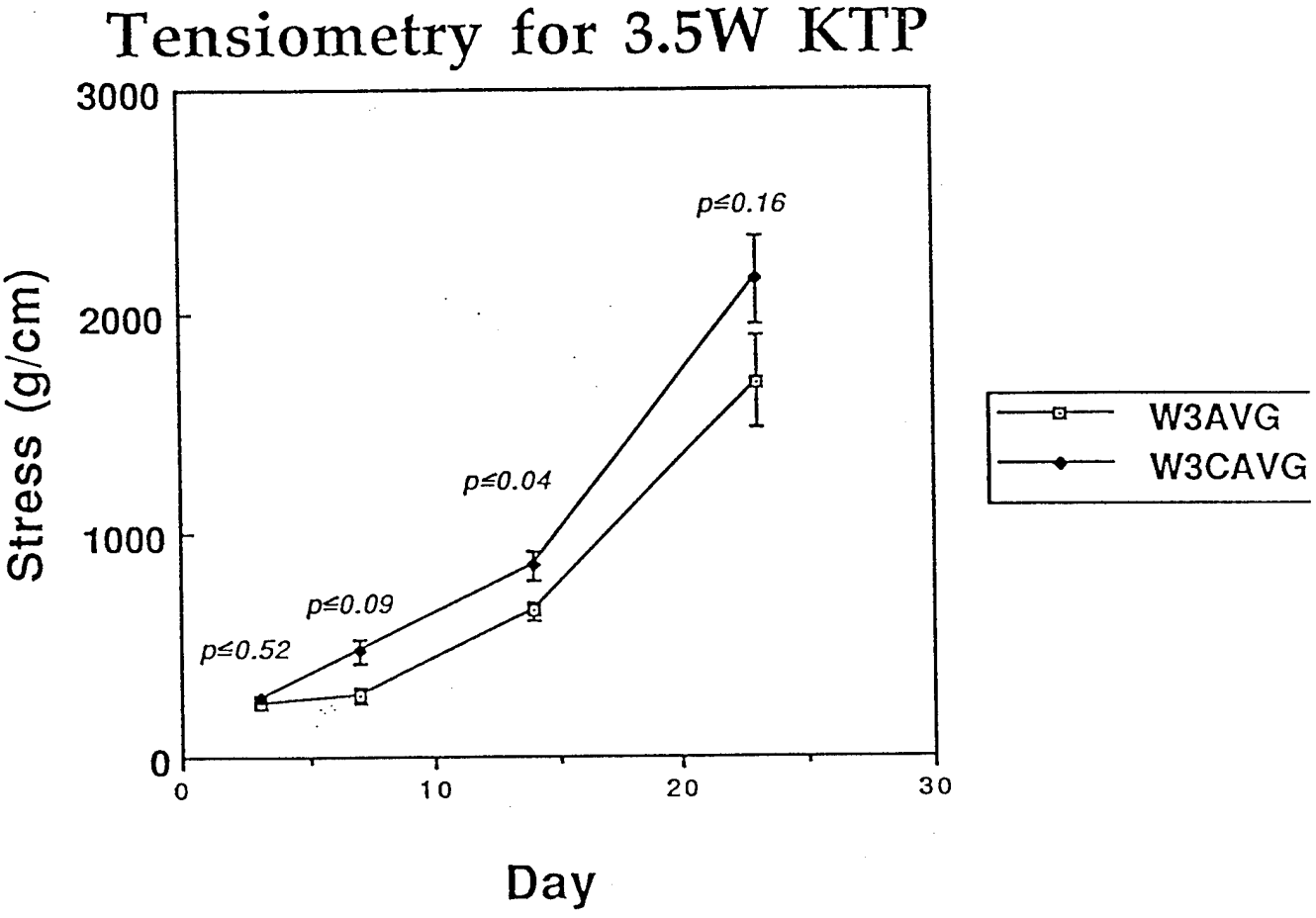
Figure 1


Tensiometry for 2.0W KTP
2000
1000
0
Stress (g/cm)
0
10
20
30
Day
p≤0.05
p≤0.01
p≤0.01
p≤0.09
W2AVG
W2CAVG

LEGEND FOR FIGURE 1

Graph illustrates temporal profile of tensiometric data for two watt irradiated incisions and their matched controls. The open boxes (W2AVG) represent mean stress values for the two watt irradiated incisions on the given days, and the solid boxes (W2CAVG) represent mean values for the two watt matched control incisions. Error bars represent standard errors of the mean. The p values were calculated using the Wilcoxon signed-rank test.

Figure 2

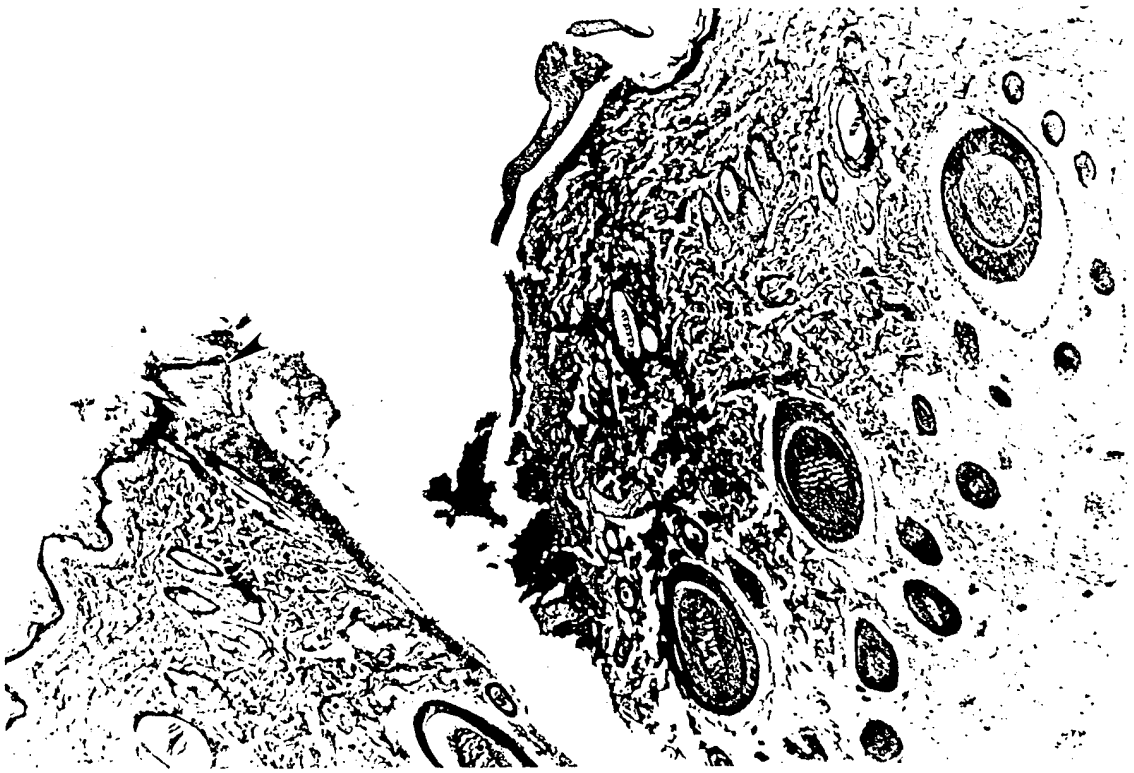

Tensiometry for 3.5W KTP
Stress (g/cm)
3000
2000
1000
0
0
10
20
30
Day
p≤0.16
p≤0.04
p≤0.09
p≤0.52
W3AVG
W3CAVG

LEGEND FOR FIGURE 2

Graph illustrates temporal profile of tensiometric data for 3.5 watt irradiated incisions and their matched controls. The open boxes (W3AVG) represent mean stress values for the 3.5 watt irradiated incisions on the given days, and the solid boxes (W3CAVG) represent mean values for the 3.5 watt matched control incisions. Error bars represent standard errors of the mean. The p values were calculated using the Wilcoxon signed-rank test.

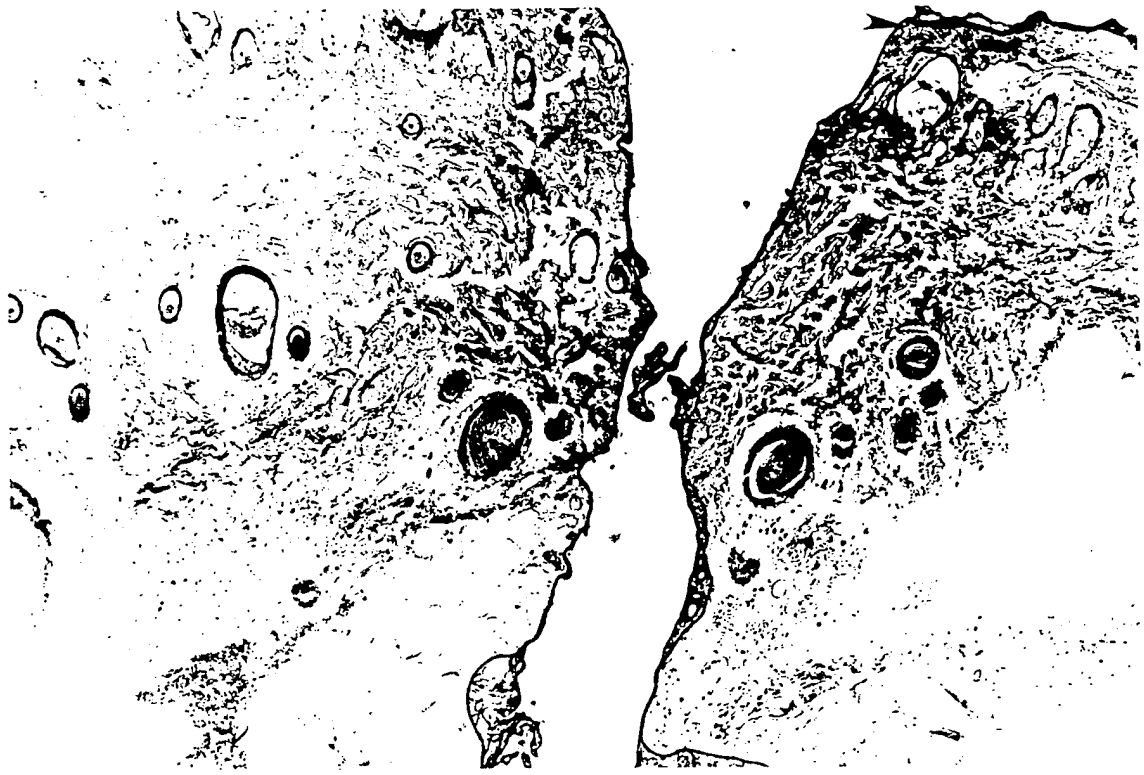
Legend for Figure 3

Figure 3: Photomicrograph of an unirradiated (control) incision harvested from an animal which was acutely sacrificed. Note the normal appearing epithelial layer (arrowhead) and subjacent dermal stroma without tissue edema. A small amount of coagulum is seen in the incisional space. Original magnification 160 X. Verhoeff's elastin stain.



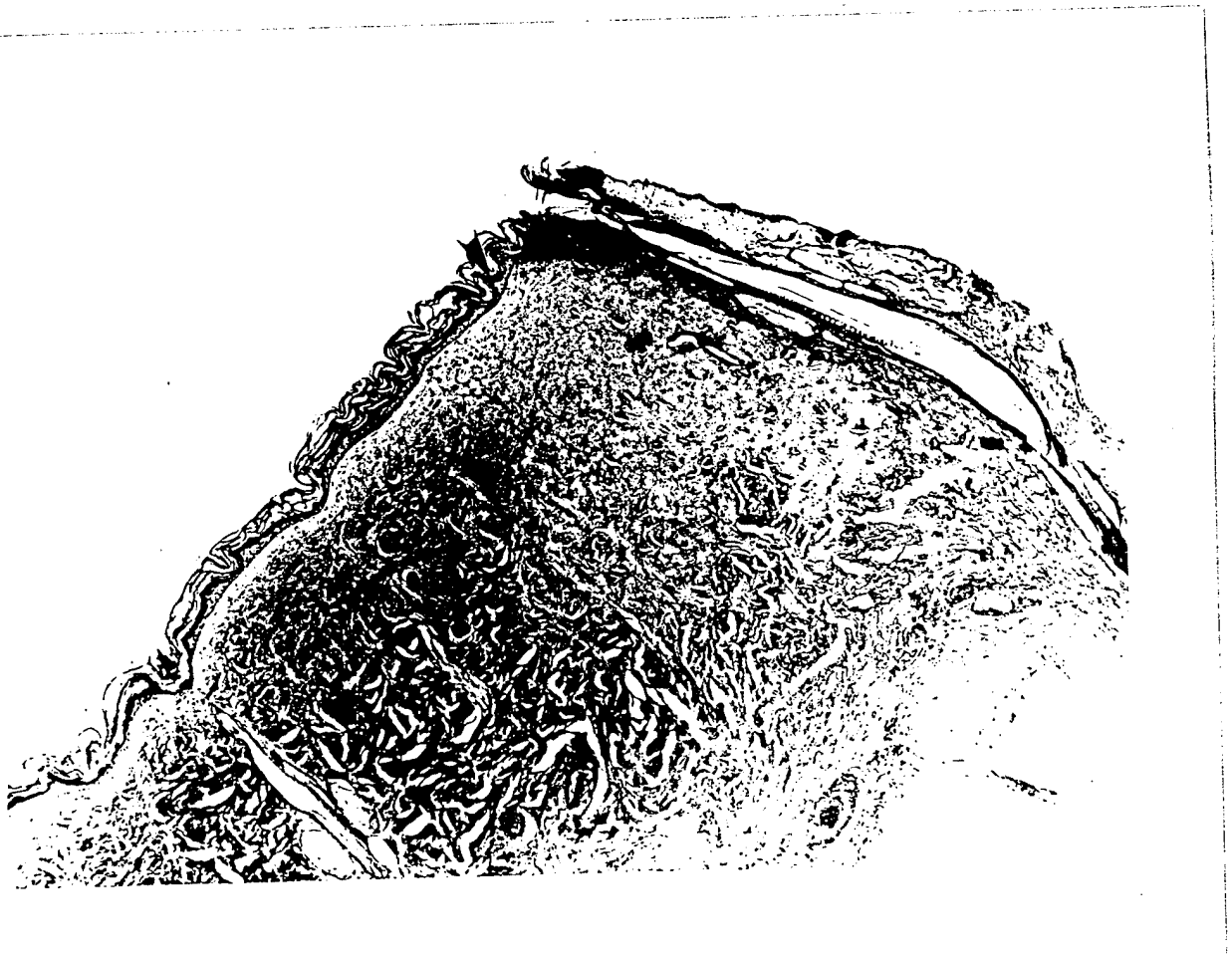
Legend for Figure 4

Figure 4: Photomicrograph of a 54J (2.0W) irradiated incision from an acutely sacrificed animal. Note again the normal appearing epithelial layer (arrowhead) adjacent to the incision, the intact dermal stroma, and the absence of tissue edema. Original magnification 160 X. Verhoeff's elastin stain.



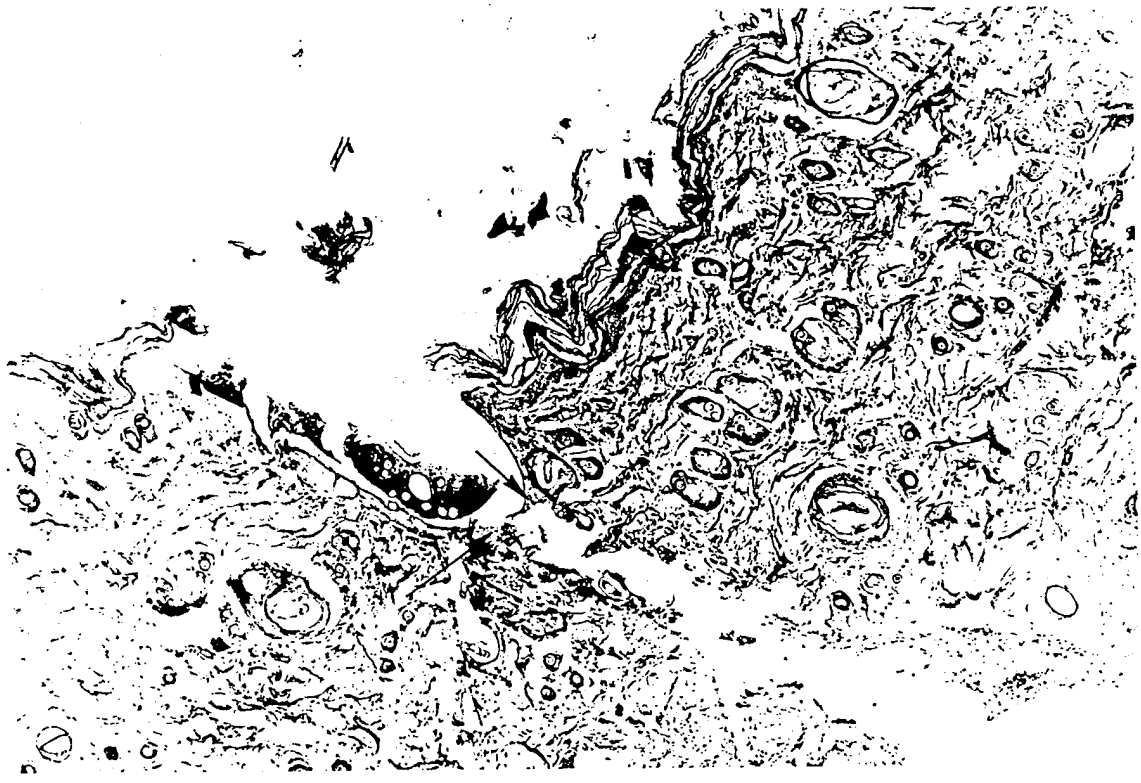
Legend for Figure 5

Figure 5: Photomicrograph of a 94.5J (3.5W) irradiated incision from an acutely sacrificed animal. Note now that although the epithelial layer remains intact (arrowhead), the superficial edge of the insision appears dark with slight eschar, and a zone of disorganized dermal collagen is seen to a depth of 0.6 mm with significant accompanying edema. Original magnification 160 X. Verhoeff's elastin stain.



Legend for Figure 6

Figure 6: Photomicrograph of an unirradiated incision at 3 days following surgery. Note that the borders of the regenerating epithelial bridge (arrows) are nearly apposed, that there are few inflammatory cells present, and that there is no apparent tissue edema. A small amount of coagulum is seen in the incision. Original magnification 160 X. Verhoeff's elastin stain.



Legend for Figure 7

Figure 7: Photomicrograph of a 54J (2.0W) irradiated incision at postoperative day 3. In comparison to postoperative day 3 control (Figure 6), note the attenuated reepithelialization (arrows indicate epithelial borders), the dense population of inflammatory cells within the dermis (arrowhead), and the augmented coagulum in the incision. Original magnification 160 X. Verhoeff's elastin stain.